



## Assessment of Bone Loss in Postmenopausal Women by Evaluation of Urinary Hydroxyproline and Serum Status of Osteocalcin

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### Abstract

Bones are in a dynamic state throughout the life. Bone metabolism is a continuous process which maintains a balance between the resorption of old and injured bone and the formation of new bone under. This continuous degradation and formation of bone is termed as bone remodeling or bone turnover, a process that occurs throughout the life. The balance between bone degradation and bone formation is altered due to aging and cessation of the menstrual cycle in women. The present study aimed at estimation of the two important markers of bone turnover, serum osteocalcin and urinary hydroxyproline in postmenopausal women and also to probe into the role of these both in assessing the altered bone metabolism. 100 women were included in the study out of which 50 were premenopausal who served as the control and 50 were postmenopausal women with mean age in years  $35.11 \pm 4.09$  and  $62.65 \pm 7.4$  respectively. Total calcium (mg / ml) and osteocalcin (ng / ml) were evaluated in the serum while hydroxyproline (mg / g creatinine) was estimated in the urine of both the groups. Significant decrease in the total calcium ( $p$  value  $< 0.001$ ) and significant increase in the serum osteocalcin level ( $p$  value  $< 0.001$ ) and urinary hydroxyproline ( $p$  value  $< 0.001$ ) was observed in the postmenopausal women. The results of the study suggested that biochemical markers like osteocalcin and urinary hydroxyproline may serve as the indicators of altered bone metabolism and therefore can be used to monitor the same in postmenopausal women subsequently helping in evaluating the risk of developing osteoporosis.

**Keywords:** Postmenopausal, osteoporosis, bone turnover, osteocalcin, hydroxyproline.

### Introduction

Metabolism of bone is a dynamic process occurring continuously throughout the life in order to maintain a balance between the resorption of old and injured bone by osteoclasts and the formation of new bone under the control of osteoblasts. This process is termed as bone remodeling or bone turnover. Bone turnover increases to high levels in women soon after menopause<sup>1</sup>. After menopause, due to the cessation of ovarian function estrogen levels decrease and beside other effects on body one major effect of estrogen deficiency is osteoporosis. This is believed to be due principally to a diminution of a direct action of estrogen on bone cells<sup>2</sup>. The two major causes of bone loss in women are deficiency of estrogen after the menopause and age related processes<sup>3</sup>. With the onset of menopause, bone loss occurs rapidly and is believed to average approximately 2% to 3% over the following 5 to 10 years, being greatest in early postmenopausal years<sup>4</sup>.

The term osteoporosis is used for the diseases that cause a reduction in the mass of bone per unit volume and is one of the dreaded afflictions of aging<sup>5</sup>. There is a close relationship between estrogen deprivation and its development. The pathogenesis of osteoporosis after menopause involves many factors such as aging, hormonal, nutritional, environmental, and genetic and life style factors etc<sup>6</sup>.

Various approaches like bone densitometry, biopsy of bone and biochemical assays provide an insight as to the knowledge of alterations in bone remodeling and bone related disorders like osteoporosis. Out of these, the biochemical assays for the skeletal metabolism hold great importance. The biochemical parameters reveal changes in bone metabolism much earlier, than the radiographic methods. They are valuable tools to evaluate the risk of accelerated bone loss in subjects, for example in postmenopausal women. Their potential in monitoring the changes in bone resorption and bone formation which would ultimately help in predicting risk of osteoporosis related fractures, needs to be explored further particularly in Indian scenario. Only a few Indian studies have probed the scope of biochemicals as markers of altered bone metabolism.

This study was aimed at evaluating the two biochemicals; urinary hydroxyproline and osteocalcin along with calcium in postmenopausal women so as to explore their role in reflecting changes in the bone metabolism.

### Material and Methods

The study was carried out at Bafna hospital and research centre, Indore. Study population comprised of 50 premenopausal women in the age group of 25 – 40 years (mean age  $35.11 \pm$

4.09) and 50 postmenopausal women in the age group of 50 – 65 years (mean age  $62.65 \pm 7.4$ ). None of the participants was on hormone replacement therapy or any alternative therapy that might affect bone turnover. All of them were non smokers and non alcoholics. All ethical measures were followed prior to the study and written consent from the study group was obtained.

Height (H) and weight (W) of all women undergoing the study were noted and Body mass index (BMI) was calculated by the formula =  $W \text{ (kg)} / H^2 \text{ (m)}$

Total calcium<sup>7</sup> and osteocalcin<sup>8</sup> were estimated in the blood samples. At the same time the urine samples were analyzed for hydroxyproline<sup>9</sup> and creatinine<sup>10</sup>.

The statistical analysis of the data involved mean  $\pm$  SD and the significance of differences in the means of the above mentioned parameters between the groups was determined by t test. For statistical analysis, Microsoft Excel was used.

## Results and Discussion

Table 1 depicts the anthropometric data of the premenopausal and postmenopausal women.

Table 2 shows the means  $\pm$  SD of the biochemical parameters as well as the significance of differences between the means of the two groups. Statistically significant decrease in the mean value of total calcium was observed in the postmenopausal women. Osteocalcin and urinary hydroxyproline were found to be significantly increased (p value < 0.001) in the postmenopausal women.

**Discussion:** Women tend to lose about 1% of bone density annually during and after menopause<sup>11</sup>. Nearly 35% of women undergo bone loss at a faster rate during the perimenopausal phase. Biochemical parameters may provide an overview as to the rates of osteoclast and osteoblast activity. Biochemical markers that reflect bone formation and resorption process, form a novel and valuable tool to monitor bone metabolism and the accelerated bone loss in postmenopausal women.

The breakdown products of bone collagen have been the recent focus of laboratory procedures designed to assess bone degradation<sup>12</sup>. One such important metabolic product of bone collagen breakdown is hydroxyproline which is excreted in urine. Urinary hydroxyproline is therefore considered as an

index of resorption of bone and a major determinant of bone status<sup>13</sup>. In our study a significant increase in the urinary hydroxyproline in postmenopausal women was found. Sachdeva et al<sup>14</sup> and Indumati et al<sup>11</sup> have reported the similar finding in their studies. The same has been confirmed by Vanita et al<sup>15</sup>. It is now well established that there is a significant increase in the urinary hydroxyproline in the peri and postmenopausal period<sup>16</sup>.

The mean osteocalcin levels were also found to be significantly higher in postmenopausal women as compared to the premenopausal women in the present study and this finding is supported by the findings of Vanita et al<sup>17</sup>. Osteocalcin is a bone matrix protein synthesized by mature osteoblasts, and constitutes about 15% of noncollagenous bone matrix proteins<sup>18</sup>. Most of the osteocalcin (80-90%) is adsorbed to bone hydroxyapatite, with a minor percentage leaking into the circulation<sup>19</sup>.

The levels of serum osteocalcin have been reported to correlate with the rate of bone formation and hence it is considered as a specific marker of osteoblast function. But since it is also released from bone matrix during bone resorption reflecting the overall turnover of bone, it is considered as a bone turnover marker.

It is a vitamin K dependent calcium binding protein produced by the osteoblasts. It has a high affinity for calcium and has a calcium dependent compact helical conformation. The carboxyglutamic acid residues of osteocalcin are capable of binding to bone matrix hydroxyapatite, thus leading to bone mineralization. Osteoporotic women deficient in calcium- and phosphorus may have a decreased rate of bone mineralization due to a reduced hydroxyapatite crystal formation. In this condition, free osteocalcin may be present in the circulation, thus explaining the increased serum osteocalcin concentration in osteoporotic postmenopausal women<sup>20,21</sup>.

Significantly higher levels of osteocalcin, bone alkaline phosphatase, and cross linked telopeptide-C (CTX) have been reported in osteoporotic females<sup>22</sup>. Pino et al<sup>23</sup> found osteocalcin to be a reliable marker of bone turnover which is useful in diagnosis and follow up of osteoporosis. Serum osteocalcin levels increase with age and women aged above 65 years have nearly 2 fold higher osteocalcin concentration as compared to those less than 44 years of age<sup>21</sup>.

**Table-1**  
**Comparison of anthropometric data of premenopausal and postmenopausal women**

Anthropometric variable	Premenopausal women	Postmenopausal women	P value
Age	35.11 $\pm$ 4.09	62.65 $\pm$ 7.4	< 0.001
BMI	22.73 $\pm$ 3.24	25.49 $\pm$ 4.9	< 0.001

**Table-2**  
**Comparison of Biochemical parameters of premenopausal and postmenopausal women**

Biochemical parameter	Premenopausal women	Postmenopausal women	P value
Total calcium (mg / dl)	9.15 ± 0.68	8.48 ± 0.44	< 0.001
Osteocalcin (ng / ml)	9.69 ± 1.44	16.7 ± 3.2	< 0.001
Urinary hydroxyproline (mg / g creatinine)	10.97± 1.41	21.14 ± 3.98	< 0.001

## Conclusion

Biochemical markers provide a dynamic measure of bone turnover and are useful in detecting changes in bone mass. These may act as indicators of altered bone turnover rate so that preventive measures may be initiated at the earliest to minimize the fracture risk in postmenopausal women due to osteoporotic changes. Assessment of bone turnover rate through biochemical markers like osteocalcin and urinary hydroxyproline could form an infallible tool to initiate the preventive measures much earlier to the onset of exorbitant bone loss and to predict fracture risk in postmenopausal women. Keeping this approach in mind we studied the markers of bone formation and bone resorption, one each along with the total calcium.

Serum osteocalcin is considered to be a specific marker of osteoblast function; however its association with the probability of fractures needs to be investigated further.

The results of urinary hydroxyproline estimation suggest that direct urinary assays to measure bone resorption have wide clinical applications in that they could be a part of screening programs to assess the osteoporotic fracture risk<sup>24</sup>. However the normal ranges for urinary excretion of hydroxyproline need to be established which would help to assess the risk of excessive bone loss and subsequent fractures in postmenopausal women.

These two and other such biochemicals of untapped potential might provide an insight into the response therapy too. With rapid advancement in technology, tests for these markers will become more reliable, more widely available and cost effective as compared to the radiographic methods.

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